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TRIPSACUM AS A POSSIBLE AMPHIDIPOID OF WILD MAIZE AND MANISURIS

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AN understanding of the origin and evolution of *Tripsacum* is important not only from the standpoint of theoretical cytogenetics but also because of the past and possible future contributions of this genus to the improvement of maize, the basic food plant of this hemisphere (Mangelsdorf, 1961).

Tripsacum has previously been considered to represent the product of collateral evolution with maize with both genera stemming from an ancient common ancestor. According to this view, the so-called diploid *Tripsacum* with 18 pairs of chromosomes is actually a polyploid species with a basic number of nine pairs. There is cytological evidence consistent with this view (Anderson, 1944; Randolph, 1955).

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³The embryo culture work which made these studies possible was accomplished by Dr. Hager as his tribute to the memory of the late Professor Edward Murray East. Dr. Hager notes that "It is satisfying that from the helpfulness he offered some 30 years ago in my lily work at the historic Bussey Institution, there developed a skill which made possible a contribution in a field that meant a great deal to him."



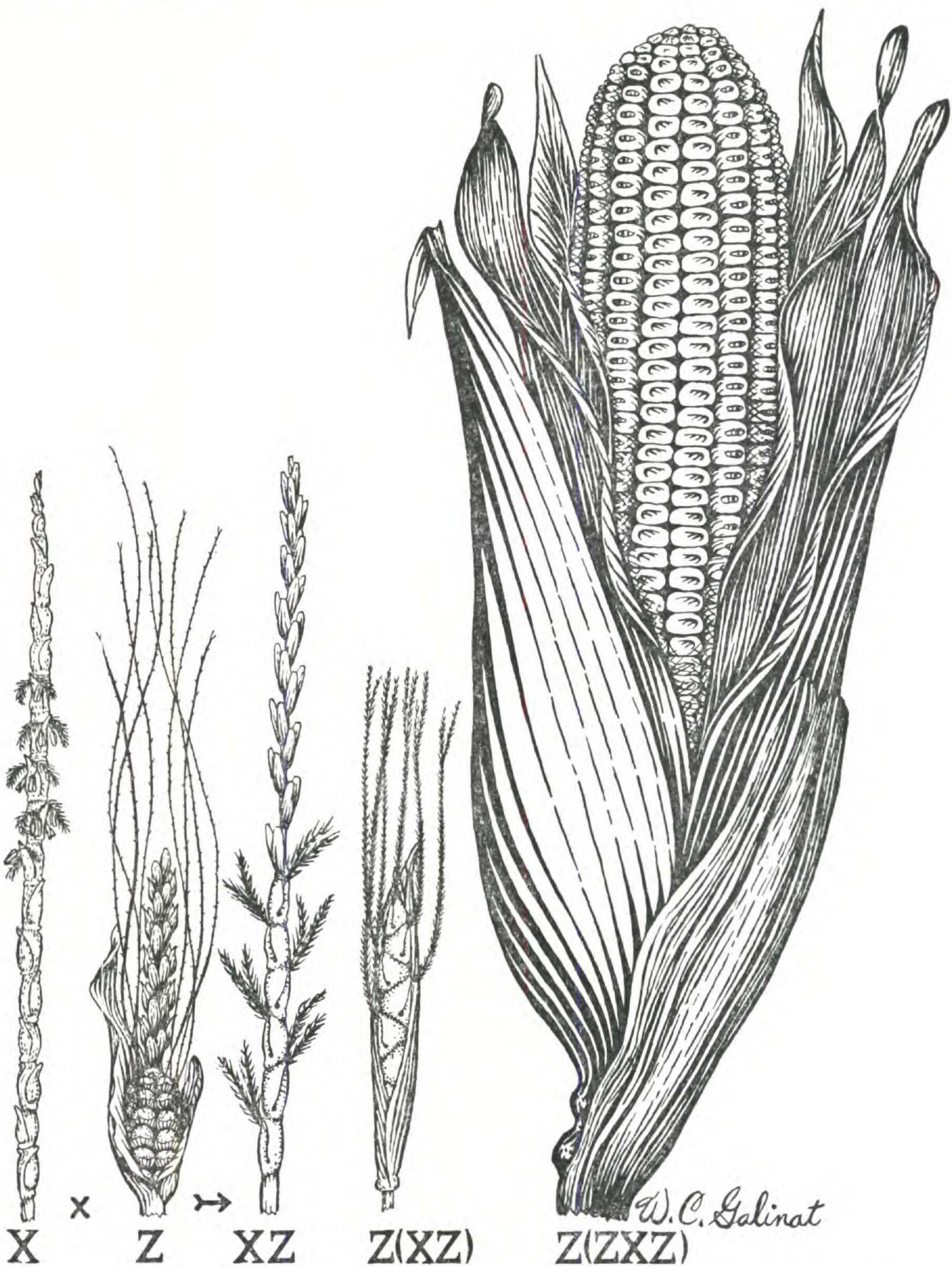
Data from studies of morphology and cytogenetics now seem to justify the presentation of a new hypothesis regarding the origin of *Tripsacum*, an hypothesis that has already been described in a preliminary statement by the senior author (Galinat, 1964). It now seems possible that *Tripsacum* has a closer relationship to maize than has previously been suspected; *Tripsacum* may be an ancient amphidiploid hybrid between wild maize and another grass, probably *Manisuris* (Plate XLVI).

MORPHOLOGICAL EVIDENCE

Even though evidence from histological characters has been essential in determining the probable phylogeny of certain grasses (Avdulov, 1931 and others), the early stage of our investigations permits consideration here of only the more traditional taxonomic characters based on gross morphology. In this case, however, they are supported by evidence from cytogenetics.

Previous taxonomical, morphological and cytological evidence suggests that *Tripsacum* is closely related to *Manisuris* and may even be a polyploid hybrid having *Manisuris* as at least one of its parents. Among the genera of the tribe Andropogoneae, *Manisuris* shows closest morphological relationship to the maize tribe, Maydeae, especially to *Tripsacum* (Weatherwax, 1935). Indeed, *Manisuris* has in the past been regarded by some taxonomists as a species of *Tripsacum* (Michaux, 1803—cf. Hitchcock, 1935). As noted by Mangelsdorf and Reeves (1939): “The most striking differences between the two genera are that *Manisuris* has perfect flowers and *Tripsacum* does not and that *Tripsacum* has the staminate and pistillate spikelets in separate portions of the inflorescence; and these differences may not be sufficient justification for considering the relationship of the two genera to be very remote.” Two of the nine species of

PLATE XLVI



A pictorial and symbolic representation of the new hypothesis suggesting that *Tripsacum*, XZ, may have originated as an amphidiploid hybrid between *Manisuris*, X, and wild maize, Z, of the type illustrated here by the reconstructed prehistoric wild maize from Tehuacán, Mexico. Teosinte, Z(XZ), and modern maize, Z(ZXZ), would, on this same notation, represent two different evolved products involving *Manisuris* introgression into maize.

Drawn by WALTON C. GALINAT

Tripsacum, *T. floridanum* and especially *T. zopilotense*, have a delicate habit of growth approaching that of *Manisuris*, while the other species of *Tripsacum* are more maize-like (maize-like), especially *T. australe* and *T. maizar*. *Tripsacum*, like maize, is confined to the New World. A preliminary survey of seven species of *Tripsacum* was made by Cutler and Anderson (1941); and since then two additional species have been discovered (Hernandez X. and Randolph, 1950). Although taxonomically *Manisuris* is often confused with *Rottboellia* and *Hemarthria*, it is clear that *Manisuris* has a world-wide distribution (Bor, 1960) and that at least five of its 12 or 13 species are native to the New World (Hitchcock, 1930, 1935).

The differentiation of *Tripsacum* into nine diverse species distributed throughout much of the New World suggests a considerable age for this genus, perhaps as much as 2,000,000 years. On the same basis, *Manisuris* would seem to be even older, an obvious requirement for a parent of *Tripsacum*, since *Manisuris* has differentiated into an even greater number of species (12 or 13) and has a world-wide distribution.

The finding of nine chromosomes in *Manisuris cylindrica* by Reeves and Mangelsdorf (1935) suggested to them that this grass was related to *Tripsacum*. In further developing this idea, Anderson (1944) states: "The cytological evidence would suggest that the 18-paired *Tripsacum* might have a cytological formula of $XXYY$ where X and Y stand for sets of 9 chromosomes, and that *Manisuris* might be XX on the same notation." On the basis of frequency of chiasma configurations per cell, Anderson postulated further "that the 36-paired varieties (of *Tripsacum*) arose as octoploids ($XXYYXXZZ$) between $XXYY$ and $XXZZ$ varieties." Anderson made no suggestion about the origin of the YY and ZZ genoms, and there is no reason to believe that the "Z" symbol in his

notation was intended to designate a genom of *Zea*. We are, however, using it so "on the same notation" (Plate XLVI).

Assuming then that *Manisuris* is one parent of *Tripsacum*, one test of whether wild maize could be the other parent lies in a comparison of the recently discovered prehistoric wild maize to these other two grasses. Such comparisons were made by Mangelsdorf and Reeves (1939) in determining if teosinte might be a derivative from maize-*Tripsacum* hybridization, and by Sarkar and Stebbins (1956) in determining what characteristics the other putative parent of the tetraploid wheats must have possessed if Einkorn were one parent. They found that *Aegilops speltoides* met virtually all of the requirements. Studies of this nature have been based on the general rule that, where an intermediate condition is possible, the presumed hybrid should show at least a tendency to be intermediate.

An examination of Table I, which compares 18 of the important botanical characteristics which distinguish *Manisuris*, *Tripsacum* and *Zea*, shows that *Tripsacum* tends to be intermediate in most cases where an intermediate condition is possible. Some detailed consideration is needed, however, to explain the functional possibilities for intermediacy and one case of apparent evolutionary elaboration of a hybrid product.

Monoecism is not only the definitive characteristic which separates *Manisuris* of the tribe Andropogoneae from *Tripsacum* of the tribe Maydeae, but it is also one of the few characteristics of *Tripsacum* which is non-intermediate between its putative parents. The monoecism of *Tripsacum* could have come as a dominant trait from wild maize, as reconstructed from archaeological remains of this grass uncovered in the valley of Tehuacán in Mexico (Mangelsdorf *et al*, 1964) and illustrated

in Plate XLVI. The dominance of monoecism might be expected, since this evolved trait is dominant within maize itself to certain recessive mutations, “anther ear” and “dwarf-1” which, among their effects, cause development of perfect flowers. Although monoecism occurs also in the Oriental Maydeae, these grasses can be ruled out as putative parents of *Tripsacum* because they are lacking in other essential characteristics, such as the thickened rachis with cupule development found in maize.

Long styles and large pollen could be derived better from maize than from any other known grass, although the wild maize which we are considering as a parent of *Tripsacum* probably had shorter styles and smaller pollen than any living race of maize, if the small size of archaeo-

TABLE I. A comparison of some of the important botanical characteristics which distinguish *Manisuris*, *Tripsacum* and *Zea*.

Characteristic	<i>Manisuris</i>	<i>Tripsacum</i>	<i>Zea</i>
1. Perfect flower <i>vs</i> monoecious	perfect flowers	monoecious	monoecious
2. Length of styles	short	intermediate	long
3. Styles, bifurcation	separate	basal fusion	fused
4. Styles, hairs	profuse	intermediate	sparse
5. Size of anthers	small	intermediate	large
6. Size of pollen	small	intermediate	large
7. Size of rachis	slender	intermediate	thick
8. Cupules in rachis	absent	present	present
9. Rachis abscission	present	present	absent
10. Rachilla to rachis	parallel	parallel (pistillate)	right angles (pistillate)
11. Glume texture	indurated	indurated	herbaceous ¹
12. Glume surface	smooth ² or sculptured	smooth	smooth
13. Spikelets binate	1 fertile, 1 sterile	staminate	staminate & pistillate
14. Phyllotaxy	distichous	distichous	polystichous
15. Leaf width	narrow	intermediate	broad
16. Height of tillers relative to main stalk	tall ³	intermediate ³	short
17. Growth habit	perennial	perennial	annual
18. Root stocks	present	present	absent

¹ Herbaceous or nearly so in archaeological wild maize.

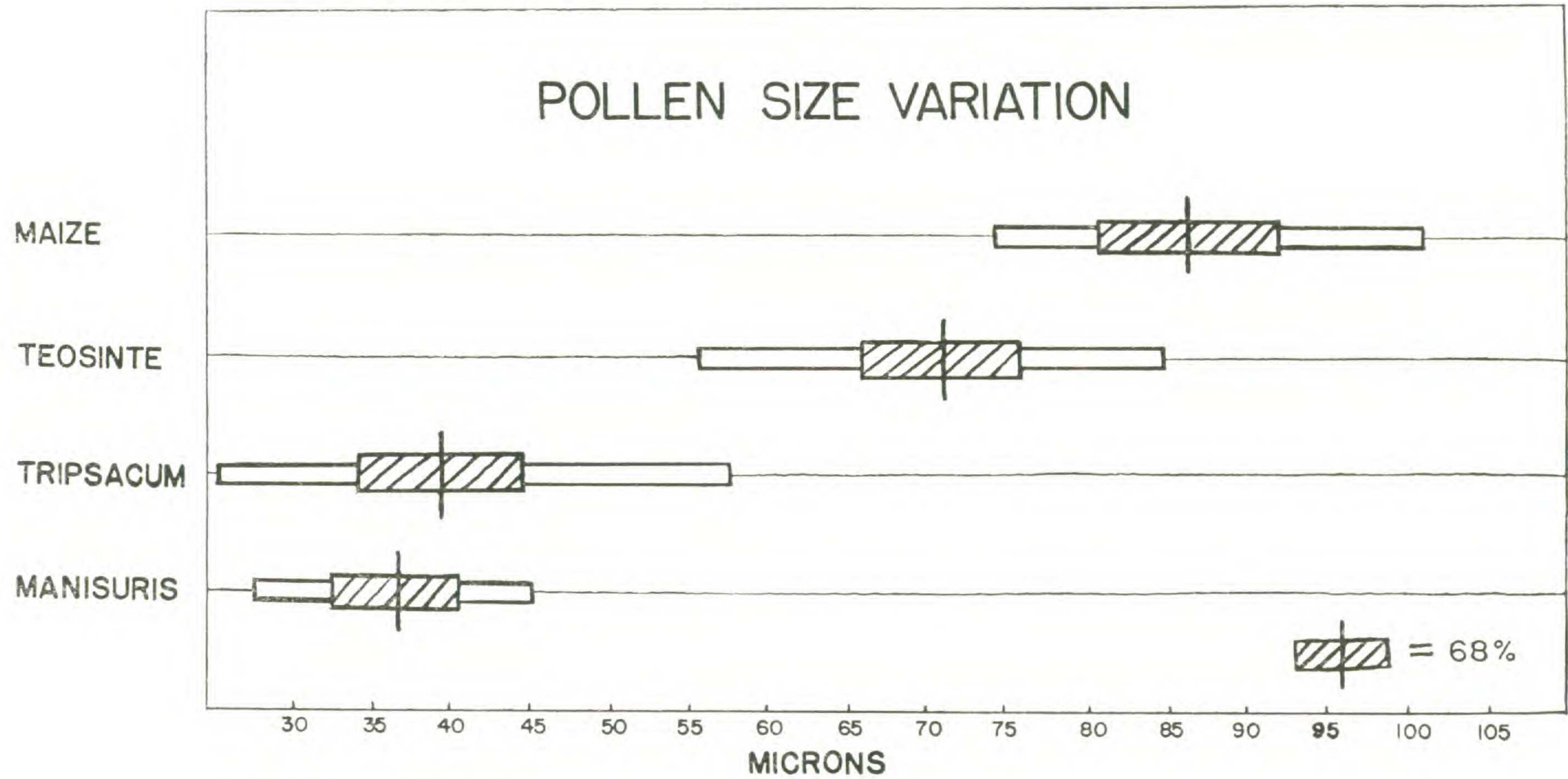
² Smooth first glumes in *M. aurita*. The other New World species are sculptured.

³ On two year old plants.

logical wild maize ears and the present-day correlation between ear size and pollen grain size are indicative (Galinat, 1961). Our data (Table II and Plate XLVII) on the variation in pollen size of three species of *Tripsacum*, show that *Tripsacum* pollen is, on the average, larger than that of *Manisuris*, but not substantially so. This might be expected, if the wild maize parent of *Tripsacum* had pollen about the size of modern teosinte or slightly smaller, since the ranges of size variation of *Tripsacum* and teosinte do presently overlap.

The first 4 or 5 mm. of style length immediately above the pistil in *Tripsacum* are fused, a feature usually not observed because it is usually concealed by the outer glume. This partial fusion may be an expression of germ-plasm from maize since this grass is unusual in having its styles (actually stigmatic branches) fused for almost their entire length. In *Manisuris*, the bifurcation of styles is complete. The styles of *Manisuris* also have numerous long hairs presenting a feather-like appearance, while those of maize usually have sparse hairs; and here, too, *Tripsacum* is intermediate though extremely variable.

The cupulate fruit case of *Tripsacum*, a device which protects the grain by means of a complicated relationship between spikelet and rachis segment, appears at first to be a sudden development in an otherwise gradual trend in the tribe Andropogoneae leading towards its formation (Galinat, 1956). Its structure may be the result of an elaboration upon a combination of features coming in from both putative parents. From maize came the unique and essential feature of the cupule, a corneous cavity with wide lateral wings that develops in the rachis immediately above the attachment point of the pistillate spikelets. The specialization of the cupule in *Tripsacum* as a functional part of the fruit case may have occurred sometime after the origin of this species. Since the wild



See Table II for details.

maize which would be one parent of the original hybrid with *Manisuris* was probably a form of pod corn and since the tunicate locus which is responsible for pod corn is dominant over the non-tunicate condition in hybrids of tunicate maize with both teosinte (Galinat, 1959) and *Tripsacum* (Galinat, unpub.), it seems probable that the original *Tripsacum* would have had the slender rachis with small cupules and the long herbaceous glumes characteristic of pod corn. Sometime during its evolutionary history, these may have been replaced by the thick rachis with large cupules and the indurated outer glumes characteristic of the non-tunicate condition and of modern *Tripsacum*.

Despite its slender rachis segments, *Manisuris* has a substitute for a cupule or a false cupule formed by the close position of sterile pedicels along alternate edges of successive rachis segments. Possessing this pedicel-formed cavity, it required only the true cupule from maize to form the cupulate fruit case characteristic of *Tripsacum*. Essentially unchanged from *Manisuris* might have come the sessile spikelets borne parallel to the rachis in a manner in which they could develop within the protective confines of a true cupule once it was introduced by maize germplasm. Also derived from *Manisuris* are the distichous arrangement of the spike and, at ma-

TABLE II. Pollen size variation in microns¹

Grass	\bar{X}	Min.	Max.	σ	n
<i>Manisuris cylindrica</i>	37.4	28	46	4.10	93
<i>Tripsacum</i> ²	40.4	26	58	5.28	489
Teosinte ³	73.0	56	86	5.10	233
Maize (Chapalote)	87.2	74	102	6.12	228

¹ From stored pollen that has been swollen with 85% lactic acid and stained with IKI solution.

² Pooled data from *T. zopilotense*, *T. floridanum* and *T. dactyloides*.

³ Pooled data from Florida teosinte and Arcelia teosinte.

turity, an extreme induration of the outer glume and rachis segment followed by rachis disarticulation with basal callus. The outer glume of *Tripsacum* and maize is smooth, while it is usually sculptured in native New World species of *Manisuris*, the exceptions being *M. aurita* of the New World tropics, and apparently certain variants of *M. cylindrica*. In any case, the smooth glume feature could have been derived as a dominant feature from maize.

Some vegetative features of *Tripsacum*, such as its broader leaves or capacity to evolve broader leaves than *Manisuris*, must have come from maize, while other vegetative features are better ascribed to *Manisuris*. The profuse tillering and perennial habit of growth in *Tripsacum* would probably have come from *Manisuris*. There is no evidence that the mere doubling of the number of chromosomes would convert an annual such as maize into a perennial such as *Tripsacum*. Certainly the perennial character would have been initially useful in the $2n$ hybrid by allowing it to persist despite sterility until a fertile $4n$ amphidiploid could arise through somatic doubling. Assuming, then, that the perennial habit did originate with *Manisuris* (because the oldest archaeological remains of maize from Tehuacán, Mexico, indicate that wild maize, like modern maize, was an annual (Mangelsdorf *et al*, 1964)) it must have been strongly dominant in its expression. All known New World species of *Manisuris* are perennial, and the rhizomatous habit is especially strong in *M. rugosa*, in which underground shoots may extend horizontally for several feet.

Even more significant than the general intermediate position of *Tripsacum* is the fact that when we use the technique of Anderson (1949) in asking the question of what source among grasses now known could make the necessary modifications in an assumed parent (*Manisuris*)

to produce a given offspring (Tripsacum), then we find that the other parent could only have been either wild maize or teosinte. Of the two, wild maize is more promising than teosinte as the putative parent.

The chief evidence that indicates wild maize rather than teosinte (*Zea mexicana* (Schrad.) Reeves and Mangelsdorf) as the non-Manisuris parent of Tripsacum is the nature of the phenotypic effects described below of two genetic types of Tripsacum chromosomes which we have experimentally superimposed as "addition monosomics" upon maize. Also the cytogenetic and morphological evidence presented by Mangelsdorf and Reeves (1939), Reeves and Mangelsdorf (1959), that teosinte is a derivative of maize-Tripsacum hybridization suggests that it is of more recent origin than Tripsacum, and, if so, could not be the parent of Tripsacum.

Although a detailed study of their phenotypes is still in the early stage, the morphological effects which we have been able to observe so far support our hypothesis as to the parents of Tripsacum. That is, there is one type of Tripsacum chromosome which is both genetically similar to maize and has "maizoid" effects which tend in the direction of wild maize. The other type seems to be genetically foreign to maize and has "manisuroid" effects tending in the direction of both teosinte and Manisuris.

The effects suggestive of the characteristics of wild maize of only two addition monosomics of the maizoid type can be included in this preliminary report. First, the Tripsacum chromosome which is known to bear at least one locus, *Lg*₁ (maize marker *liguleless*₁), and probably many more, including *Gl*₂ (maize marker *glossy seedling*₂) and *Ws*₃ (maize marker *white sheath*₃) as found by Maguire (1962) has effects on the ear which we consider tend toward wild maize. Although the reduction

in plant height and possibly the increase in tillering (Table III), as also observed by Maguire (1956), may be effects only from a chromosomal unbalance, the combination of longer internodes in the rachis (measured as thicker kernels) with shorter ears bearing a lower number of kernel rows would not result from just a general reduction of vigor. In the background of modern maize, long internodes in the rachis are associated with longer ears, even under conditions of stunting, but in primitive maize such as Confite Morocho of Peru (Grobman *et al*, 1961) and in the archaeological wild maize of Tehuacán, Mexico (Mangelsdorf *et al*, 1946), relatively long internodes in the rachis are associated with tiny eight-rowed ears. The data (Table III) show that there was no overlapping in the range of variation for ear length and almost none for kernel rows and kernel thickness. The one 10-rowed ear which occurred in the 21-chromosome plants was actually eight-rowed at the tip of the ear and, thereby, showing a tendency to be eight-rowed like the other ears bearing the extra chromosome. None of the 20-chromosome plants were eight-rowed. Thus, the Trip-

TABLE III. Phenotypic effects of the *T. dactyloides* counterpart for chromosome 2 when added to maize.¹

Character	20-chrom			21-chrom.		
	\bar{X}	Min.	Max.	\bar{X}	Min.	Max.
Plant height cm.	96.0	75.0	120.0	90.5	73.0	110.0
No. of tillers	2.8	2.0	3.0	4.3	4.0	5.0
Ear length cm.	14.2	13.5	17.0	10.5	7.7	12.0
Kernel rows	11.5	10.0	12.0	8.1	8.0	10.0
Kernel thickness mm. ²	3.6	3.3	3.8	4.2	3.7	4.7

¹ Since the population is small (16 plants), maximum and minimum are given rather than standard deviations.

² Averaged from 10 consecutive kernels in the central region of each ear.

sacum homeolog¹ or counterpart for chromosome 2 has several wild-maize-like effects on the ear.

The *Tripsacum* homeolog for maize chromosome 9 produces a staminate tipped ear, a characteristic of wild maize as reconstructed in Plate XLVI. These changes suggestive of wild maize are not associated with increases in glume or rachis induration, as they might be had they been caused by either teosinte or *Manisuris* germplasm. Also both of these maizoid isolates from *Tripsacum* as well as others bearing several genetically marked chromosomes from *Tripsacum* are usually completely female fertile and at least partially if not completely male fertile.

In sharp contrast are the effects of the "manisuroid" isolates which have extra chromosomes not markable by any of the seven marker genes of WMT maize (described later) which, if *Manisuris* is one parent of *Tripsacum*, may represent the chromosomes originally from *Manisuris*. The manisuroid isolates tiller more profusely, have narrower leaves, smaller spikelets, smaller kernels, increased induration of rachis and glumes, less specialization between the staminate and pistillate glumes and are partially female sterile and usually completely male sterile. The tendency for the staminate and pistillate glumes to be more alike through a reduction in the usual development of wings on the pistillate glumes and a reduction of the usual increased elongation of the staminate glumes, is of significance because with the perfect flowered condition of *Manisuris*, there is no pressure for glumes which are specialized into two shapes, one for protecting kernels, the other for protecting anthers.

¹ A term sometimes spelled *homoeologue*, used first by Huskins (Am. Nat. 75: 329-344, 1941) to designate the genetically similar chromosomes in the hexaploid wheat genom.

CYTOGENETIC EVIDENCE

The conception of *Tripsacum* as an amphidiploid of wild maize and *Manisuris* originated from the rather surprising results of a segregation of *Tripsacum* chromosomes from a hybrid of *T. dactyloides* with a unique multiple tester stock of maize which has recessive marker genes on seven chromosomes (1, 2, 3, 4, 7, 8, 9). This "WMT" stock of maize was developed by Mangelsdorf some years ago expressly for the purpose of crossing with *Tripsacum*. Its development started in Texas on a background of the variety "Mexican June." Later, after being moved to Massachusetts where earlier maturity proved to be necessary, germplasm from two northern inbreds, P39 and A158, was added. The original hybrid of *T. dactyloides* with this multiple tester was actually produced on a vigorous line cross between two inbred strains of the WMT stock. The principal purposes in making this hybrid with *Tripsacum* were: (1) to determine whether *Tripsacum* carries dominant alleles of the maize recessives; (2) to determine whether *Tripsacum*, having almost twice as many chromosomes as maize, carries the dominant alleles in duplicate in some cases; (3) to identify cytologically the *Tripsacum* chromosomes which carry the dominant alleles.

A hybrid plant, obtained by the embryo culture work of Dr. Hager, exhibited none of the seven recessive characters introduced from maize but proved to be completely sterile. Consequently, colchicine treatment to double the chromosome number was required in order to produce a tetraploid hybrid. This proved to have only about 50 per cent female fertility (Galinat, 1961), although virtually full female fertility of the F_1 on the $2n$ level occurs in maize-*Tripsacum* hybrids involving another species, *T. floridanum* (Galinat, 1962). Back-

crosses of the F₁ hybrid to the WMT maize stock produced triploid plants having 20 maize and usually 18 *Tripsacum* chromosomes. The second backcross to WMT yielded a population of 92 plants segregating for varying numbers of extra *Tripsacum* chromosomes, ranging from one to thirteen, of which seven were marked by dominant genes.

Our genetic analysis (Galinat, Mangelsdorf and Chaganti, 1963) of the transmission frequencies of the seven dominantly marked *Tripsacum* chromosomes indicated a negative answer to the second question: *i.e.*, does *Tripsacum* carry the dominant alleles in duplicate? The frequencies of these dominants were similar, averaging 32.2 per cent with the exception of J₁ which was present in almost twice this frequency (Table IV). We now know that at least part of the high frequency of J₁ was the result of a poor expression of *j*₁ which is characteristic of this gene. The progeny of another generation of backcrossing to WMT of one plant classified as J₁ proved to be all *j*₁, while another similar backcross progeny proved to be all green, perhaps because of the presence of one of the *R* alleles which is known to be associated with a factor inhibiting the expression of *j*₁ (Emerson, Beadle and Fraser, 1935).

The average transmission frequency of 32.2 per cent for each dominantly marked chromosome (except J₁) was interpreted as a deviation from the expected 50 per cent on random segregation of a single chromosome. This was

TABLE IV. Genetic data for transmission rate to 92 WMT maize plants of seven dominantly marked *T. dactyloides* chromosomes derived from a maize-*Tripsacum* BC₁ hybrid.

	Maize Chromosome						
	1	2	3	4	7	8	9
Dominant from <i>Tripsacum</i>	Bm ₂	Lg ₁	A ₁	Su ₁	Gl ₁	J ₁	Wx
Frequency of Dominants, %	34.5	23.9	31.6	28.2	38.0	60.5	37.0

the first clue that there was only one homeolog in *Tripsacum* for each of the maize chromosomes.

Confirmation of this indication followed the chromosome counts made by Chaganti for individual plants in about a third of this segregating population. First, one of the genetic addition monosomics for Wx^T (62-586-20) which bore an ear segregating Wx^T kernels at the frequency (32 per cent) that had been attributed to single homeolog transmission, was in fact found cytologically to have only one extra *Tripsacum* chromosome. Secondly, chromosome counts in many of the other plants revealed the presence of various numbers of unmarked *Tripsacum* chromosomes in addition to those which represented homeologs of the seven recessively marked chromosomes in WMT maize (Table V). Since three of the 10 maize chromosomes were unmarked by recessive genes, the counts of extra *Tripsacum* chromosomes have to be considered accordingly. For example, plant 62-582-27 carried 13 extra *Tripsacum* chromosomes and seven dominant markers leaving not less than three nor more than six unmarked addition chromosomes; Plant 62-588-65 carried 12 addition chromosomes with only three dominant markers leaving six to nine unmarkable chromosomes; Plant 62-586-43 carried five addition chromosomes with no dominant markers, leaving two to five of the unmarkable type.

If we assume that there is in *Tripsacum* a genom of nine unmarkable chromosomes and a genom of nine markable chromosomes for which two markers are lacking in the WMT stock, then we can calculate the number of unmarked chromosomes to be expected in the population of 163 extra *Tripsacum* chromosomes listed in Table V. This turns out to be 100; the actual number found was 85. The value of Chi-square for the ratio is 5.82 which represents a *P* value of less than .02.

TABLE V. Segregation of genes and chromosomes in the testcross progeny of the triploid hybrid [(*bm1, lg1, a1, su1, gl1, j1, wx* × *T. dactyloides*) × *bm1, lg1, a1, su1, gl1, j1, wx*].

Plant Number	Number of Chromosomes	Number of extra Tripsacum chromosomes	Dominants from Tripsacum	Number of unmarked Tripsacum chromosomes excluding J
62-580- 8	24	4	Su, Lg, (J) ¹	2
- 9	23	3	Su, Lg	1
-10	27	7	Su, Lg	5
62-582-16	21	1	Wx	0
-18	24	4	Wx	3
-20	21	1	Wx	0
-22	22	2	Wx, (J)	1
-23	20	0	None	0
-25	28	8	Su, Wx, A, Gl, Bm, (J)	3
-26	29	9	Su, Wx, A, Gl, Lg, Bm	3
-27	33	13	Su, Wx, A, Gl, Lg, Bm, (J)	7
-29	25	5	Su, Wx, Lg, (J)	2
-31	29	9	Su, Wx, Bm	6
-32	22	2	Su, Wx	0
62-586-41	22	2	(J)	2
-42	26	6	Bm	5
-43	25	5	None	5
-45	23	3	Lg, (J)	2
-47	23	3	(J)	3
-49	27	7	Wx, A, Gl	4
-51	28	8	Wx, Gl, (J)	5
-59	24	4	Wx, A, (J)	2
-60	24	4	Wx, A, (J)	2
-61	25	5	Wx, Lg	3
62-588-65	32	12	A, Gl, Bm	9
-68	27	7	A, Gl, (J)	5
-71	25	5	Gl, (J)	4
-72	24	4	Gl	3
-77	25	5	Gl, Bm	3
-81	22	2	A	1
-84	23	3	A	2
-86	23	3	A	2
-89	22	2	Lg, (J)	1
-92	25	5	Lg, (J)	4
Totals		163	63	100
Theoretical *			54	109

¹ See text for unreliability of *J* phenotypic expression. The total at the bottom of the column excludes *J*.

* On the assumption that there is a genom of nine unmarkable chromosomes and a genom of nine markable chromosomes for which three markers are lacking.

Knowing from the progenies of some of the plants listed in Table V that the classification for *J* has been inaccurate in some cases, we have made another calculation excluding *J* as one of the markers. The observed ratio of marked and unmarked chromosomes is now 100:63 and the theoretical ratio is 109:54. Chi-square for this ratio is 2.24 which represents a *P* value of 0.14.

The closeness of the fit between the theoretical and observed ratios of marked and unmarked *Tripsacum* chromosomes in this population is regarded as good, indeed almost conclusive, evidence that *Tripsacum* contains two distinct genomes. One of these is so remotely related to maize that it contains no dominant counterparts of the maize marker recessives tested so far and presumably its nine chromosomes are not homeologs of maize chromosomes. This is the genome which we assume to have been derived from a genus of the tribe Andropogoneae, probably *Manisuris*. We are, accordingly, designating this the "manisuroid" or X genome. The other genome is much more closely related to the maize genome, since it does carry dominant alleles of the maize recessive. Furthermore, some of these chromosomes have a similar gene content to their maize homeologs. For example, Maguire (1962) has found that the genes *Ws₃*, *Lg₁*, *Gl₂* which in maize occur on the short arm of chromosome 2, are also linked together on the long arm of one of the *Tripsacum* chromosomes, although according to our recent unpublished data, the *V₄* gene on the other arm of maize chromosome 2 is located elsewhere in our "Bussey clone" of *T. dactyloides* (2n of Kansas). Maguire (1961) found also that this *Tripsacum* chromosome segment competes successfully about 5 per cent of the time in synapsis with maize chromosome 2 to the exclusion of its partner. Furthermore, she found that pollen bearing this extra chromosome from *Tripsacum*

functioned in direct competition with pure maize pollen about 42 per cent of the time. Thus, this particular *Tripsacum* chromosome is in several respects the counterpart of modern maize chromosome 2, although, it is shorter in length and may not have the counterpart of the V_4 locus on this particular chromosome in maize.

Our own data likewise show that the *Tripsacum* homeolog of maize chromosome 9 which carries a dominant allele of *wx* also carries dominant alleles of *sh*₁ and *bz*, two other recessives whose loci occur on chromosome 9.

Thus, even the cytogenetic data so far available suggest strongly that the second genom is much more closely related to maize than the *X* or "manisuroid" genom. Accordingly, we are designating this as the "maizoid" or *Z* genom. This genom may have been derived from a wild maize not too different in its characteristics from the prehistoric wild maize described by Mangelsdorf *et al* (1964) or it may have come from the remote ancestor of that maize.

Another means for the cytological corroboration for the presence of the postulated *X* and *Z* genoms within *Tripsacum* is by comparing the frequency of various types of synaptic relationships within haploid maize, *Z*, and haploid *Tripsacum*, *XZ*, to that of the *F*₁ maize-*Tripsacum* hybrid, *Z* (*XZ*). A model for their expected behavior on the basis of this hypothesis can be set up and tested against the observed behavior. Such a model would stipulate low intragenomic pairing of the *Tripsacum* chromosomes and considerable intergenomic pairing between maize and *Tripsacum* chromosomes when the chromosomes of the two species are brought together in the same cell.

The behavior of chromosome synapsis was studied in haploid maize and in hybrids of maize and *Tripsacum floridanum* (a close relative of *T. dactyloides*) (Chaganti

and Galinat, 1964) and their backcross progenies (Chaganti, 1964). The objective of this study was to score the frequency of associations which are a consequence of effective synapsis (synapsis followed by exchange, reflected as chiasmatic associations at metaphase) and homologous or homeologous synapsis in haploid maize (reflected as side-by-side associations at metaphase, Person, 1955; Riley and Chapman, 1957), in the haploid genom of *Tripsacum* and in the F_1 maize-*Tripsacum* hybrid. If the pairing frequency in one or both the species or their sum is equal to or greater than that of the hybrid, then most of the pairing in the hybrid would be autosyndetic and not reflective of intragenomic affinities. If, on the other hand, the pairing in the hybrid is higher than that of the parents individually or even that of their sum, then such an increase in pairing could only result from some intragenomic or allosyndetic associations (Riley and Chapman, 1957).

The haploid maize used for this type of comparison was found in the backcross progeny of a triploid hybrid of maize and *Tripsacum floridanum*. Although no haploid *Tripsacum* plants as such were available or have ever been discovered, the essential information for our purposes was obtained from observations made on a haploid genom of *Tripsacum* isolated within a triploid hybrid which also contained two genoms of maize in each cell. Since the two genoms of maize pair with each other rather than compete with the *Tripsacum* chromosomes, any synaptic relationships within the *Tripsacum* genom are expressed, except when confused by rare but apparent maize-*Tripsacum* interchanges which occurred in the parental F_1 hybrid. Furthermore, any other exchanges between the non-homologous *Tripsacum* chromosomes occurring in the F_1 hybrid will be apparent (if the exchange chromatids are transmitted to the egg develop-

ing into the triploid hybrid) as extra bivalents in the triploid hybrid (Maguire, 1964). Thus, the behavior of *Tripsacum* chromosomes in the triploid hybrid may be taken to represent the intragenomic affinities within the *Tripsacum* genom. In Table VI, the mean per cell of chiasmatic and side-by-side association found at meta-anaphase in haploid maize, a haploid genom of *Tripsacum* (in the triploid hybrid) and the F₁ maize-*Tripsacum* hybrid are presented (also see Plate XLVIII).

TABLE VI. Mean per cell of chiasmatic and side-by-side associations and their range (in parenthesis) in haploid maize, a haploid genom of *Tripsacum* and the F₁ maize-*Tripsacum* hybrid. The rest of the chromosomes occurred as unpaired univalents or end-to-end associations of various kinds and frequencies. It is assumed (Person, 1955; Riley and Chapman, 1957) that the later type of associations do not imply homologies or homeologies.

	Mean per cell of chiasmatic trivalents	Mean per cell of chiasmatic bivalents	Mean per cell of s-s assns.	Remainder of chromosomes
Haploid maize	0.00	0.06 (0 to 1)	0.28 (0 to 2)	9.32
Haploid genom of <i>Tripsacum</i>	0.06 (0 to 1)	0.20 (0 to 1)	0.20 (0 to 1)	17.07
Haploid maize plus haploid genom of <i>Tripsacum</i>	0.06	0.26	0.48	27.34
F ₁ maize- <i>Tripsacum</i> hybrid	0.00 (0 to 4)	2.28 (0 to 4)	0.69 (0 to 2)	22.04

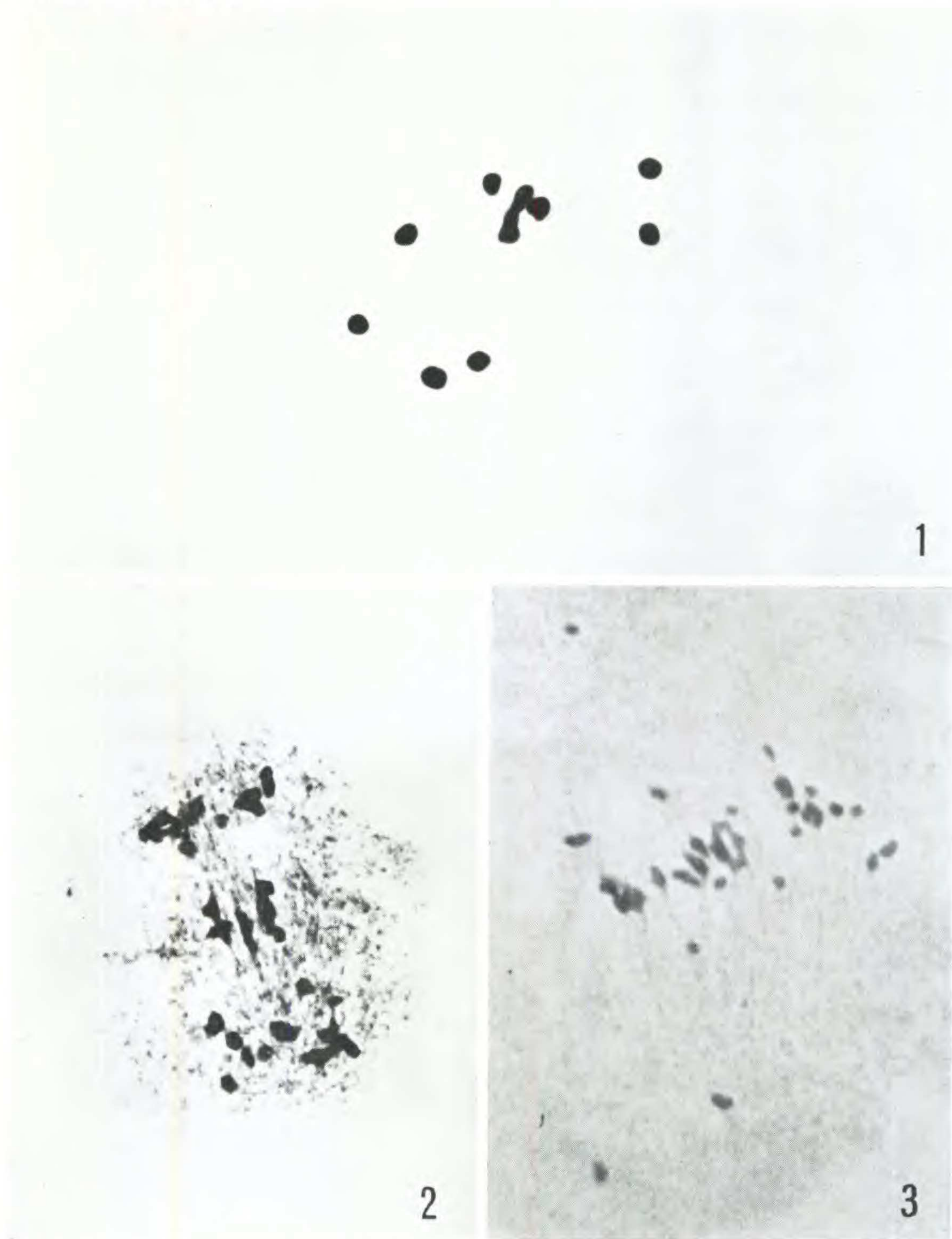
The above data clearly demonstrate that the mean synapsis is higher in the F₁ hybrid of maize and *Tripsacum* compared to the pairing in the parents individually or together and hence most of it must be allosyndetic. In this connection, it is of interest to refer to certain observations on chromosome exchanges in maize-*Tripsacum* hybrids made by Maguire (1964) in a study of chromosome pairing in the backcross progenies of maize-*Tripsacum* hybrids. Maguire studied two triploid hybrids, one

of which showed 10 maize bivalents and 18 *Tripsacum* univalents consistently. In the other, out of 34 cells examined, at metaphase, 27 (or 79.4%) showed two trivalents each, nine (or 26.5%) showed one trivalent each, and 24 (or 70.6%) showed an additional *Tripsacum* bivalent besides the 10 maize bivalents. She attributed the trivalents to maize-*Tripsacum* exchanges in the F_1 hybrid, while the extra *Tripsacum* bivalent was assumed to have arisen from interchanges within the *Tripsacum* genom. Thus, Maguire's data, like ours, show a rather low intragenomic exchange either in the F_1 or the triploid hybrid. In the one hybrid in which prior exchange is reflected it seems that maize-*Tripsacum* exchanges are more frequent than *Tripsacum*-*Tripsacum* exchanges.

The foregoing data and discussion clearly demonstrate three facts: (1) there is low intragenomic pairing within haploid maize, (2) there is also low intragenomic pairing within the haploid genom of *Tripsacum*, and (3) pairing in the maize-*Tripsacum* hybrid is allosyndetic.

If this allosyndetic synapsis involves all of the *Tripsacum* chromosomes such that the entire *Tripsacum* genom shows synaptic affinities to the maize genom, then one would assume that *Tripsacum* is a polyploid with two similar sets of chromosomes. In such a case, the chances for the members of a given *Tripsacum* genom to pair and exchange segments with their homologs or homeologs in the maize genom would be equal to their chances of pairing with the members of the second *Tripsacum* genom. This would be reflected as a higher (than observed) frequency of extra bivalents from *Tripsacum* in the triploid hybrid. Furthermore, in the F_1 hybrid there would be at least some proportion of trivalent pairing. Since no such trivalents were encountered in the F_1 hybrid of this test, the evidence indicates that the two genoms of *Tripsacum* are dissimilar.

PLATE XLVIII



Chromosome association in haploid maize, maize-Tripsacum hybrid and the triploid hybrid. 1, Meta-anaphase of haploid maize showing 8 univalents and one bivalent. 2, Meta-anaphase of maize-Tripsacum hybrid showing three bivalents and 22 univalents. 3, Metaphase I of the triploid hybrid showing a ring of four maize chromosomes (probably due to exchange between two non-homologous chromosomes in the F_1) 8 maize bivalents and 18 Tripsacum univalents. 1, 2 and 3, 800 \times .

DISCUSSION

The hypothesis presented here regarding the ancient origin of *Tripsacum* from an amphidiploid hybrid of wild maize and *Manisuris* is a testable one. We shall continue with experiments designed to test it in various ways, and we hope that others will also be interested in doing likewise by all means, including, for example, the use of techniques sometimes referred to as "chemical taxonomy." Virtually all of the morphological and cytological data which we have been able to evaluate so far is consistent with the hypothesis. An exception yet to be explained, however, is the fact that if the 10 pairs of chromosomes in modern maize are combined with the nine pairs of *Manisuris*, we might expect it to produce an amphidiploid with 19 pairs rather than the 18 pairs of *Tripsacum*. The most plausible explanation at the moment is that one maize chromosome has been lost, probably in the $2n$ hybrid.

The past and possible future contributions of *Tripsacum* germplasm to the evolution of maize should be more completely evaluated. Genes for the perennial habit, the immunity to some of the major fungal diseases affecting maize (Malm and Beckett, 1962), increased resistance to heat and drought, stiffer leaves and stalks (Mangelsdorf and Reeves, 1939), early flowering and longer internodes above the ear position (Galinat, 1963) and increased yielding capacity and improved chlorophyll characters (Reeves and Bockholdt, 1964) are probably but a few of those which we might wish to transfer from *Tripsacum* to maize.

Mangelsdorf (unpub.) has pointed out that because the apparent wild maize genom in *Tripsacum* has been subjected to some very rigorous environments for long periods of time, it may possess genes of great economic

value, some of which can undoubtedly be transferred to maize (*cf.* Reeves and Bockholt, 1964). Thus, maize breeders might select *Tripsacum* for introgressive purposes from the region in which they are interested. In this connection, Beckett and Lambert have already conducted a state-wide survey of *Tripsacum* in Illinois for possible maize breeding purposes (Lambert, 1963).

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